AMPHOTERICIN B NEPHROTOXICITY IN PATIENTS WITH CRYPTOCOCCAL MENINGITIS.

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A Dissertation submitted to the Faculty of Medicine, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Medicine. Johannesburg 2017.

DECLARATION

I Maroka Kafofora Gerald declare that this Dissertation is my own work. It is being submitted for the Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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(Signature of candidate)

_____day of _____20_____in_____

Dissertation: Amphotericin B Nephrotoxicity in patients with Cryptococcal Meningitis.

MMed Internal Medicine

ABSTRACT

Introduction

Cryptococcal infections, which occur mainly as opportunistic infection in immunosuppressed patients, contributes 20% to HIV-related mortality especially in resource-limited regions like Sub-Saharan Africa with the number of infected people at 6,100,000 in 2012 in South Africa according to UNAIDS.[7] Currently, Amphotericin B deoxycholate forms the cornerstone of all treatment protocols for cryptococcal meningitis, especially in these regions. Amphotericin B deoxycholate, the original formulation, is associated with significant risk of nephrotoxicity with up to 80% of those receiving the drug showing an increase in serum creatinine level, a marker of renal function.[10] The availability of the safer lipid formulations of amphotericin B are limited by their costs, more so in resource-limited regions.[8]

However, in South Africa data on Amphotericin B associated nephrotoxicity is sparse.

Aims

The aim of this study is, therefore, to describe nephrotoxicity regarding incidence and outcome in patients with cryptococcal meningitis at Klerksdorp/Tshepong Hospital Complex on Amphotericin B administered per local protocol.

Objectives

- To define the frequency of acute kidney injury (AKI) on amphotericin B in this cohort of patients who had enough data for analysis.
- To assess whether electrolyte abnormalities predict AKI by multivariable analysis in the cohort in this study
- To describe overall outcome based on:
- Mortality.
- Progression to chronic kidney disease, as evidenced by un-resolving of renal dysfunction for three months or more.[3]
- Need for acute dialysis.
- Morbidity related to known sequela of cryptococcal meningitis.

Methods

This is a retrospective observational review of amphotericin B nephrotoxicity in patients with cryptococcal meningitis at Klerksdorp/Tshepong Hospital Complex from July 2013 to June 2014 to describe amphotericin B deoxycholate associated nephrotoxicity.

Name and file numbers for patients who tested positive for cryptococcal meningitis, by either cryptococcal latex antigen or India ink on cerebrospinal fluid, were obtained from national health laboratory service (NHLS). 165 names and file number where retrieved but only 53 had sufficient data for the study.

Results

The files reviewed showed that 83% developed acute kidney injury with 5.66% progressing to chronic kidney disease. However, only 28 participants had a record of follow-up attendance. Mortality was 18.87% with no statistically significant association with acute kidney injury. However, mortality was higher in the acute kidney injury group (9:1). None of the study participants received dialysis. There was also a statistically significant association between being on antiretroviral treatment (Tenofovir-containing regimen) and acute kidney injury. The dose of amphotericin B was positively associated with the development of acute kidney injury. 90.56% of the participants received fluid supplementation per protocol recommendations during amphotericin B therapy.

Conclusion

Amphotericin B is a nephrotoxic drug with the risk of AKI significantly increased in patients on Tenofovir, necessitating ART regimen review before initiating therapy. The treatment protocol should be applied more strictly including correct dose, electrolyte and fluid supplementation to minimize nephrotoxic effects. Cryptococcal meningitis in HIV-infected patients on treatment has a mortality of 18.87% in this cohort which was lower

compared to available literature, which estimates mortality at 30 to 40% on amphotericin B based protocols. [12, 16, 21]

This cohort did not reveal any other statistically significant occurrence of long-term complications of cryptococcal meningitis.

PREFACE

This study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand- ethics clearance protocol number: M141193

LIST OF ABBREVIATIONS

ADH	Anti-diuretic Hormone
AKI	Acute kidney Injury
ATP	Adenosine Triphosphate
AIDS	Acquired immune deficiency syndrome
ART	Anti-retroviral therapy
CSF	Cerebrospinal fluid
CKD	Chronic kidney disease
CI	Confidence interval
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HOD	Head of department
kg	Kilogram
NHLS	National health laboratory service
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
RR	Relative risk
RRT	Renal replacement therapy
SIRS	Systemic inflammatory response syndrome
UNAIDS	United Nations Programme on HIV and AIDS
WHO	World health Organization

ACKNOWLEDGEMENTS

The project was made possible with the help of the following people to whom I would like to dedicate a heartfelt appreciation. My supervisor Professor Graham Paget, HOD Nephrology at Charlotte Maxeke Johannesburg Academic Hospital.

I also want to show appreciation for the support and encouragement I received while I was doing my general medicine rotation at Tshepong hospital from the department as a whole, especially Professor I. Variava, Professor B. Luke and Dr. F.R. Sigauke, Dr K. Tshiovhe and Dr M Mukansi, HOD Critical care at Helen Joseph Hospital.

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1. LITERATURE REVIEW

1.1. General Introduction

HIV/AIDS (Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome) is a pandemic that affects humans in all spheres of existence, with some describing it as a human rights issue more than just a viral infection, because of its impact on all aspects of human existence and functioning. According to UNAIDS, HIV infections were at 6,100,000 in 2012 in South Africa and still increasing.[7] The clinical syndrome that results from the manifestation of the HIV infection, AIDS, is characterized by immunosuppression that leads to opportunistic infections. Cryptococcal meningitis is one such opportunistic infection contributing significantly to the HIV-related mortality, especially in third world regions like Sub-Saharan Africa. [5, 15, 18]

The clinical syndrome of AIDS is associated with high mortality related to such opportunistic infections, which are a challenge to treat due to the immunosuppressed state[4]. Starting antiretroviral (ART) therapy early is also associated with the risk of immune reconstitution inflammatory syndrome (IRIS), with a potential fatal outcome due to the systemic inflammatory response syndrome (SIRS) as the recovering immune system aggressively reacts to the under treated or unrecognized cryptococcal infection.[16]

Such patients are often treated with potent drugs that may have severe adverse effects, which may further worsen the prognosis and complicate their management. Amphotericin B forms the cornerstone in the management of cryptococcal meningitis. However, nephrotoxicity is one of this therapy's potentially severe adverse events, with up to 80% of patients receiving the drug, having elevated blood creatinine levels as a result of treatment.[10] When acute kidney injury happens in resource-poor regions, renal replacement therapy (RRT) may not be available even when clinically indicated due to high cost. [29]

This study looks at patients with cryptococcal meningitis on amphotericin B to investigate potential associated nephrotoxicity. The patients are treated based on a treatment protocol that is developed to minimize the occurrence of adverse events and

where they do occur, to detect and manage them early. This study will also give an indication of whether we are doing enough for these patients or indicate whether we need to invest more in developing safer treatment options like lipid formulations with low nephrotoxic effects of Amphotericin B [8], and the need for RRT.

The aim of this study is, therefore, to describe nephrotoxicity regarding incidence and outcome in patients with cryptococcal meningitis at the Klerksdorp/Tshepong Hospital Complex receiving Amphotericin B administered per local protocol.

1.2. Cryptococcal Meningitis

1.2.1 Introduction

Cryptococcal meningitis is the most important cause of meningitis in adults in sub-Saharan Africa, contributing 20% to yearly HIV/AIDS-related mortality[5,15, 34] and accounts for 45% of causes of meningitis in adults in Harare Zimbabwe.[20] It therefore follows that cryptococcal meningitis should be mastered by clinicians in this field, and such a need cannot be over emphasized.[35] However, literature and research on this subject in South Africa is limited, especially when examining the nephrotoxic effects of amphotericin B.

1.2.2 Etiology

Cryptococcal meningitis is caused by infection of the meninges, with or without encephalitis, by a yeast-like fungus, most importantly *Cryptococcus neoformans* or less commonly *Cryptococcus gatti*.[12] There are two serotypes of *C. neoformans* A and D, while *C. gatti* has B and C serotypes. [14, 19] Cryptococcosis is spread by aerosolized particles from avian excreta especially pigeons, with *C. gatti* found in most types of arboreal species.[22]

1.2.3 Pathophysiology

Infections seem to occur as early as childhood and although there is no evidence to support latent subclinical infection, it is a strong hypothesis, as clinical infection becomes apparent in hosts with significantly depressed immunity with no evidence of recent exposure. This is especially common in HIV infection when the CD4 count is less than 200 cells/ml. [4]

After inhalation, the Cryptococcus particles make their way to the lungs where they rarely cause a clinical infection, but seem to disseminate by an unknown mode to the cerebrospinal fluid or meninges, though haematogenous spread is thought to be the likely mode.[4,12]

The virulence characteristics of *C. neoformans* include the capsule that has antiphagocytic urease and phospholipase enzymes that enhance survival in tissues. [12] *C. neoformans* activates the immune system through the production of copious quantities of the polysaccharide antigen. The detection of these polysaccharide antigens in serum by latex antigen tests forms the basis of the diagnosis of cryptococcosis. [4]
Resistance characteristics of Cryptococcus neoformans include regulation of structure and composition of the cell membrane sphingolipids. [22]

1.2.4 Clinical Manifestations

Cryptococcal meningitis should always be considered in patients who present with a chronic headache and a background of immunosuppression. *Cryptococcus gatti* may cause predominantly pulmonary disease also in immunocompetent hosts.[23] Cryptococcal infection can affect any organ system with no specific clinical findings,[13] but the central nervous system and pulmonary involvement are more common.[4] The clinical manifestations include headache with or without fever, lethargy, low level of consciousness with or without signs of meningeal irritation, cranial nerve palsies and visual impairment, commonly in immunosuppressed patients. [20, 37]

Pulmonary manifestations are non-specific and may also follow a chronic course presenting with chronic productive cough and chest pains, which may progress to respiratory failure.[16]The chest radiographs may reveal areas of radio-opacities caused by

granulomatous masses, which may be difficult to differentiate from pneumonia, tuberculosis and cancer.[23, 24]

Skin manifestations include; papules, plaques, purpura, vesicles, tumor-like lesions and rashes.[4,16]

1.2.5 Diagnosis

The diagnosis of cryptococcal meningitis is made by examination of the cerebrospinal fluid which will show mononuclear pleocytosis and high protein content. Confirmation tests include cryptococcal antigen latex tests that detect the cryptococcal polysaccharide antigen in the cerebrospinal fluid.[4] The latex cryptococcal antigen titer kits are both sensitive and specific for cryptococcosis both in serum and cerebrospinal fluid.[37] These latex cryptococcal antigen titer kits are commercially available, and include the Immy and Meridian enzyme immunoassay kit and pronase-containing latex kits with sensitivity of 97% and specificity of 93-100%.[36]

The demonstration of cryptococcal antigenemia precedes the clinical disease by about six weeks with the World Health Organization (WHO) recommending screening of HIV patients with CD4 less than 100 cells/ml. [15] The WHO further recommends prompt lumber puncture for analysis, with measurement of opening cerebrospinal fluid (CSF) pressure, and both serum and CSF cryptococcal antigen titer for early diagnosis of cryptococcal meningitis.[31]

The diagnosis of pulmonary Cryptococcus may be difficult due to the non-specific clinical presentation and radiological findings. Investigations include serum antigen detection, sputum culture, and direct biopsy for histopathology.[23, 24]

The Southern African HIV Clinicians' Society drafted and released the updated recommendations for the screening of cryptococcal meningitis in December 2012.[11] Active screening for HIV-positive patients with CD4 of less than 100 cells/ml is

recommended also taking into account the presence or absence of suggestive symptoms of active cryptococcal meningitis including headache, convulsions, neurological deficits and impaired level of consciousness to decide between prophylaxis and full treatment of the disease.[20, 21]

1.2.6 Treatment

Treatment regimens for cryptococcal meningitis do not differ significantly, with almost all having amphotericin B as the main drug. Flucytosine or fluconazole may be considered for use during the induction phase.[16] Fluconazole is continued during the consolidation phase and for secondary prophylaxis.[37] WHO recommendations for treatment of cryptococcal meningitis include first the use of combination therapy with amphotericin B and flucytosine, and in the absence of flucytosine, fluconazole is added instead of flucytosine.[31] Flucytosine is included in the WHO Lists of Essential Medicines' core list.[32] The South African national guidelines (standard treatment guidelines and essential medicine lists 2012) and the Klerksdorp/Tshephong complex guidelines however do not include flucytosine due to high cost of the drug and the drug is not registered in South Africa. This would also be the case in most resource limited regions like the Sub-Saharan Africa.[12, 30]

Treatment options are tailored depending on the type of infection. Pulmonary cryptococcosis in an immunocompetent host is treated with fluconazole oral 400mg daily for six to twelve months.[24] In extra-pulmonary cryptococcal disease, amphotericin B is the preferred drug given during the induction phase, followed by consolidation and prolonged or life-long maintenance/prophylactic fluconazole with or without flucytosine.[16] Please see the attached protocol for treatment of cryptococcal meningitis from Klerksdorp/Tshepong Hospital Complex as appendix B.

The combination of amphotericin B and flucytosine is shown to be superior to amphotericin B and fluconazole or amphotericin B alone.[12] Flucytosine in combination with amphotericin B improves fungal clearance and reduced 10-week mortality by up to 39%.[30, 34] Lipid formulations of amphotericin B also have an additional advantage in

limiting renal dysfunction and also associated with early clearance of the cryptococcal infection.[16]

HIV-infected patients are given fluconazole for prophylactic purpose in patients with CD4 of less than 200 cell/mL until the CD4 counts improve to more than 200 cells/mL. However, initiation of ART early on has a risk for immune reconstitution inflammatory syndrome (IRIS) with a potentially fatal outcome, thus further complicating the management of cryptococcal infection in HIV-infected patients.[4,16] Lipid formulations of Amphotericin B can also be used in those with renal dysfunction.[3]

It is thus recommended that in HIV-associated cryptococcal meningitis, the initiation of ART should be delayed by six weeks to reduce the occurrence of paradoxical cryptococcal meningitis IRIS that affects 14 to 30% of those successfully treated for cryptococcal meningitis.[18, 21] Cryptococcal meningitis IRIS has a high mortality rate especially when it is associated with encephalitis.[16]

The HIV Clinicians' Society in view of the findings of the Strategic timing of antiretroviral treatment therapy (START) and early antiretroviral treatment and/or early isoniazid prophylaxis against tuberculosis in HIV-infected adults (TEMPRANO ANRS 12136) studies, recommends antiretroviral treatment initiation in all HIV-infected patients. There should be no delay initiating those with CD4 below 350 cells/mm^3 and those with CD4 of less than 200 cells/mm^3 should be fast tracked for initiation on ART.[33]

1.2.7 Prognosis

Cryptococcal meningitis in HIV-infected patients has a poor prognosis, with 100% mortality in patients who do not receive antifungal treatment [21]. In patients with HIV-associated cryptococcal meningitis on amphotericin B based treatment regimens, mortality rates are as high as 30 to 40%.[12, 16, 21]

There is no one determinant of prognosis, but low level of consciousness and hyponatraemia seem to be associated with a poorer outcome.[20]

1.3. Amphotericin B

1.3.1 Introduction

Amphotericin B is a naturally occurring polyene macrolide produced by *Streptomyces nodus*, which has been modified to reduce its toxicity with flucytosine that may be added to reduce the therapeutic dose of amphotericin B and thus its toxicity.[27, 53] It is a drug of choice for most life-threatening systemic mycoses.[8]

Amphotericin B is a very effective drug in treating cryptococcal meningitis [27] and forms the cornerstone of all protocols/regimens in its management, from international to local centers, including the Klerksdorp/Tshephong Hospital complex in North West province of South Africa.

1.3.2 Mechanism of action and spectrum of activity

Amphotericin B acts in a complex and multifaceted way, including binding to ergosterols in plasma membranes of sensitive fungal cells making them porous to the electrolytes like potassium, and auto-oxidation resulting in free radical formation by the action of catalase and glutathione peroxidase leading to cell death.[26] The spectrum of activity includes *Candida albicans, Histoplasma capsulatum, C. neoformans, Coccidioides immitis, Blastomyces dermatidis* and strains of Aspergillus.[43] Resistance is conferred by decreased ergosterol content in the fungal membranes, overexpression of tryparedoxin peroxidase leading to protection against oxidative stress, and amphotericin B efflux by the ATP-binding cassette transporters in the cell membrane.[8, 53]

1.3.3 Pharmacodynamics

Amphotericin B is insoluble in water, and as a result sodium deoxycholate is added to the injectable solutions given by intravenous route.[8] The direct intrathecal route for therapy of meningitis is rather dangerous and therefore not recommended.[8] Amphotericin B sodium deoxycholate poorly crosses the blood-brain barrier in the absence of inflammation, unlike its lipid formulations, but crosses the placenta.[51, 52] The drug is eliminated through bile and urine and, therefore, requires dose adjustment in liver and kidney disease.[8]

1.3.4 Side effects

Amphotericin B's most important side effect is nephrotoxicity, and has a maximum recommended dose of 1.5mg/kg/d. The drug affects glomerular filtration, creatinine clearance, and magnesium and sodium handling by the kidneys.[43] Normocytic normochromic anaemia may result from reversible erythropoietin suppression induced by renal dysfunction.[51] Hypotension from a shock like reaction associated with hypokalaemia has been described.[8] Thrombophlebitis may result at the injection site.[29]

Amphotericin B related nephrotoxicity can be fatal if not recognized and treated early,[8] though stopping treatment may leave the patient at the mercy of this fatal disease. This further burdens the health budget if the nephrotoxicity results in severe acute kidney injury requiring dialysis, which is one of the most expensive treatment interventions of modern medicine, especially in resource limited regions like Sub-Saharan Africa. Patients who develop acute kidney injury necessitating dialysis have three times higher mortality rates compared to those who do not, with a mortality rate of 76% in those who are not dialyzed compared to 56% in those who are dialyzed when required.[29]

Mechanisms of Amphotericin B nephrotoxicity include alteration of intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy in a dose-related pattern.[3, 28] Amphotericin B also does to a lesser degree bind to cholesterols in tubular cells resulting in porous membranes, causing leaking of magnesium and potassium in proximal tubular epithelial cells, causing proximal tubular necrosis, as well as the back leak of hydrogen ions in distal tubular cells resulting in distal renal tubular acidosis.[26]

The electrolyte abnormalities associated with amphotericin B nephrotoxicity include hypokalemia, hypomagnesemia, hypocalcaemia and hyponatraemia especially if associated with proximal tubulopathy.[43, 28] Hypernatremia may occur when there is associated dehydration.[3]

Amphotericin B associated increases in serum urea and creatinine have been shown to occur in as many as 80% of patients receiving the drug, with doubling of the serum levels of urea and creatinine in 40 to 60% of those receiving it.[28, 29] These increases are dose related with about 44% of those receiving a cumulative dose of more than 4g progressing to chronic renal failure compared to only 17% of those who received a cumulative dose of less than 4g.[10]

In 1964, Butler et al. evaluated inulin and para-amino-hippurate (PAH) in patients who received a cumulative dose of Amphotericin B of 1.536g to 3.11g, and showed a decrease in renal blood flow and glomerular filtration rate during treatment, which lasted for about five months in five of the patients after stopping the drug.[10]

The histo-pathological changes associated with amphotericin B nephrotoxicity also seem to be dose related with both glomerular and tubular changes. The glomerular histological changes include thickening of the basement membrane, hypercellularity, fibrosis and hyalinization. Tubular manifestations may be focal or generalized predominantly affecting the ascending limb of the Loop of Henle, the distal convoluted tubules and the collecting ducts. Amphotericin B causes degeneration and atrophy in the affected sites. Amphotericin B also causes nephrocalcinosis in the proximal and distal convoluted tubules resulting in divalent calcium ion found intraluminally, intracellularly, and interstitially, but mostly at the corticomedullary junction.[8, 10]

Lipid formulations available are abelcet, amphoteric and ambisome with better tolerability, and reduced nephrotoxicity demonstrated through clinical trials like the meta-analysis by Aguirre and Hamid[40] as discussed below. It would seem thus that the only drawback against lipid formulations of Amphotericin B is increased cost.[6, 29, 40]

Aguirre and Hamid [40] published a meta-analysis on the 23rd November 2015 in which they included all randomized controlled trials at the time that compared amphotericin B sodium deoxycholate with liposomal amphotericin B, to assess the effects of amphotericin B sodium deoxycholate versus liposomal amphotericin B, on renal function. Twelve studies with 2298 participants were included, ten of which were analyzed with 2172 participants. The primary outcome was increased serum creatinine level to double the baseline value. The study results showed that liposomal amphotericin B is significantly safer than amphotericin B sodium deoxycholate regarding serum creatinine increase (RR 0.49, 95% CI 0.40 to 0.59). Infusion-related adverse events were also found to be significantly reduced in the liposomal amphotericin B group.

Leenders et al [39] conducted a randomized trial in HIV-infected patients to receive Ambisome (liposomal amphotericin B) 4 mg/kg daily or standard amphotericin B 0.7 mg/kg daily in a 1 to 1 randomized manner for three weeks, with both followed by fluconazole 400 mg daily for seven weeks. The results of the 28 evaluable patients from the study showed no statistically significant difference in the clinical outcome but significantly lower nephrotoxicity in the liposomal arm despite the higher dose.

Acute renal failure is defined as a rapid deterioration in glomerular filtration rate (GFR) resulting in accumulation of metabolic wastes like urea and creatinine, classified into pre-renal, intrinsic and post-renal.[2,54]

The KIDGO 2012 guidelines define acute kidney injury as follows [3]:

- Increase in serum Creatinine by >26.5 umol/L within 48 hours; or
- Increase in serum Creatinine to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior seven days; or
- Urine volume <0.5 ml/kg/h for 6 hours

However, in pre-renal failure, the reduction in GFR is a physiologic compensatory mechanism to maintain intravascular volume by renal auto-regulatory mechanisms. Failure to adequately restore renal blood flow may lead to irreversible damage if not corrected timeously, leading to acute tubular necrosis.[2, 54]

Measures to reduce the nephrotoxic effects of amphotericin B include intralipid and other pharmacological agents like furosemide and Mannitol, which have not been proven to be consistently effective. Prolonging the infusion rate of amphotericin B over four hours, as in the attached protocol (Appendix B), is another way to reduce the nephrotoxic effects of the drug. Careful attention is important in managing risk factors for amphotericin B nephrotoxicity which include: daily and cumulative dose of amphotericin B given, dehydration, preexisting renal dysfunction and concomitant use of other nephrotoxic drugs.[29]

1.4 Fluconazole

Fluconazole is an antifungal drug that acts by inhibiting the synthesis of ergosterols, which are important components of the fungal cell membrane. It has good tissue penetration including the CSF, with or without inflammation.[8]

When compared to the other azoles like miconazole and ketoconazole, fluconazole is highly active against *Cryptococcus neoformans* with excellent bioavailability after oral administration, it crosses the blood-brain barrier, and is 80% renal excreted.[38]

Fluconazole is added to the regimen mainly for prophylactic purposes after the treatment phase of cryptococcal meningitis. The spectrum of activity of fluconazole includes C. *neoformans, Candida albicans,* and *Coccidiomycosis*. The drug is excreted unchanged in urine with no nephrotoxic properties, but dose adjustment in renal disease is required. Side effects include nausea.[8]

1.5 Flucytosine.

Flucytosine, first produced in 1957, is a synthetic drug, a fluorinated analog of cytocine with no antifungal activity in its original form. The antifungal activity of flucytosine derives from its metabolite 5-fluorouracil after deamination of flucytosine by cytocine deaminase, which humans do not possess. 5-fluorouracil causes miscoding of RNA and inhibits DNA synthesis.[8, 34]

Resistance to flucytosine in Cryptococcus neoformans is found in 1-2% of cases and develops by one of two mechanisms: mutations that affect cytocine deaminase and increased amounts of pyrimidines that compete with the fluorinated antimetabolites of flucytosine.[34]

Important adverse effects include bone marrow suppression, hepatotoxicity and teratogenicity when used during pregnancy.[34]

The combination of amphotericin B and flucytosine is shown to be superior to amphotericin B and fluconazole or amphotericin B alone in the treatment of cryptococcal meningitis [12]. Flucytosine in combination with amphotericin B improved fungal clearance and reduced ten-week mortality by up to 39%.[30, 34]

The superiority of combination therapy with flucytosine and amphotericin B is recognized by WHO, and flucytosine now forms part of the WHO Essential Drug List.[31, 32]

1.6 Anti-retroviral treatment and renal dysfunction.

The introduction of highly active antiretroviral therapy (HAART) has significantly impacted the fight against HIV infection, with life expectancy in infected individuals improving by decades. The commonly used drugs against HIV infection include: nucleoside reverse transcriptase inhibitors(NRTI), non-nucleoside reverse transcriptase inhibitors(NNRTI), entry/fusion inhibitors and protease inhibitors.[3, 56]

Nucleoside reverse transcriptase inhibitors show significant elimination by renal clearance including both glomerular filtration and tubular secretion. The urinary concentration of Tenofovir is as high as 70% after intravenous administration and up to 35% after chronic oral administration. Emtricitabine has elimination by renal excretion of 85% of the administered drug dose. These drugs require dose modification in individuals with impaired renal function.[56, 65]

Tenofovir is administered as the prodrug Tenofovir disoproxil fumarate as a once-a-day tablet or in combination with other ARV's, as one of the commonly used NRTI's. Tenofovir's nephrotoxic effects include proximal tubulopathy, with findings consistent with acute tubular necrosis on histopathology that manifest as Fanconi's syndrome with the following associated abnormalities: normal anion gap metabolic acidosis, hypophosphatemia, hyperphosphaturia, hypokalaemia, hypouricemia, urinary tubular protein waste, glucosuria, and aminoaciduria.[3, 55] Tenofovir also causes reduction in aquaporin 2 channels resulting in nephrogenic diabetes insipidus.[55]

Tenofovir induced proximal tubulopathy and nephrogenic diabetes insipidus may progress to acute renal injury, with less than half of the affected individuals recovering their baseline renal function once the drug is withdrawn. Risk factors associated with Tenofovir induced nephrotoxicity include; poor baseline renal function, concomitant use of other nephrotoxic drugs, age, diabetes mellitus, hypertension, and advanced HIV infection.

Non-nucleoside reverse transcriptase inhibitors, such as the commonly used efavirenz and nevirapine, mainly undergo elimination by hepatic metabolism.[56] Renal excretion in this category of drugs amounts to less than 3% and dose adjustment in individuals with impaired renal dysfunction are not necessary.[56]

Protease inhibitors are mainly eliminated via hepatic metabolism with renal excretion accounting for 10% for indinavir and less than 5% for other drugs in the same class. Protease inhibitors do not require dose adjustment in individuals with impaired renal function.[61]

Enfuvirtide, the available entry/fusion inhibitor, has limited data available in individuals with impaired renal function, but seems not to be affected by impaired renal function.[56]

1.7 Electrolyte disturbances and nephrotoxicity.

The susceptibility, and therefore the severity, of acute kidney injury from medications depends on a number of factors including the following; dose of the nephrotoxic drug, concomitant use of other nephrotoxic drugs, dehydration, preexisting renal dysfunction, sepsis, multisystem conditions like hypertension and pre-existing electrolyte disturbances.[29, 66]

Electrolyte disturbances that influence nephrotoxicity of drugs such as gentamycin include; hypercalcemia, hypokalemia, and phosphate depletion. Potassium chloride loading is protective against gentamycin induced nephrotoxicity, likely via stimulation of the Na,K-ATPase activity and decreasing gentamicin cellular uptake. This is however problematic in clinical settings due to potentially fatal effects of hyperkalemia, like cardiac arrhythmias.[65, 66]

Hypokalemia and hypercalcemia may result in diabetes insipidus. When diabetes insipidus occurs in patients with impaired thirst mechanism or in patients who have reduced fluid intake for any reason, such as severely ill patients with cryptococcal meningitis, dehydration may result. This will cause increased risk of developing nephrotoxicity when such nephrotoxic drugs, such as amphotericin B are administered without correction of the fluid depleted state caused by the diabetes insipidus.[43,45,67]

Hypokalemia induced diabetes insipidus is related to the insensitivity or resistance of the aquaporin-2 receptors to the endogenous anti-diuretic hormone (ADH). This results in impaired urine concentrating ability and as a result, fluid preservation.[67] Hypokalemia is also associated with reduced renal blood flow mediated resulting from reduced systemic vascular resistance and renin mediated increased renal vascular resistance.[68]

Chronic hypercalcemia causes ADH insensitivity by disruption of the renal concentrating mechanism through calcification and fibrosis of the interstitial tissues. This insensitivity to ADH results in poor urine concentration and fluid preservation, and thus, diabetes insipidus. If the fluid balance is disrupted by impaired fluid intake, dehydration results which potentiates the toxic effects of nephrotoxic drugs like amphotericin B.[4,68]

2 CHAPTER TWO: STUDY DETAILS

2.1. Aims and Study Objectives

The aim of this study was to describe nephrotoxicity regarding incidence and outcome in patients with cryptococcal meningitis at Klerksdorp/Tshepong Hospital Complex on Amphotericin B administered per local protocol between 01/07/2013 and 30/06/2014.

The objectives:

- To define the frequency of AKI on amphotericin B in this cohort of patients who had enough data for analysis, as defined by KIDGO [3].
- To assess whether electrolyte abnormalities predict AKI by multivariable analysis in the cohort in this study.
- To describe the following outcomes:
    - Mortality.
    - Progression to chronic kidney disease, as evidenced by non-resolving of renal dysfunction for three months or more [3].
    - Need for acute dialysis.
    - Morbidity related to known sequela of cryptococcal meningitis.

2.2. Study design:

This was a retrospective, observational study with descriptive and analytical components looking of patients with cryptococcal meningitis on amphotericin B at Klerksdorp/Tshepong hospital complex from 01/07/2013 to 30/06/2014.

2.3. Inclusion criteria:

- Adult patients diagnosed with cryptococcal meningitis on cerebrospinal fluid.
- Adult patients treated with amphotericin B between 01/07/2013 and 30/06/2014 at Klerksdorp/Tshepong complex, for cryptococcal meningitis.
- File available for analysis at Tshepong Hospital.

- End points:

    - Normal renal function tests after completion of amphotericin B.
    - Normal renal function tests at follow-up before three months' post completion of amphotericin B.
    - Consistently abnormal renal function tests beyond three months after stopping or completion of amphotericin B (chronic kidney disease).
    - Death before any of the above is achieved.

The extent of renal injury was classified according to the KIDGO staging of AKI. Long-term complications of cryptococcal meningitis were noted.

The protocol: amphotericin B is administered at a dose of not more than 1mg per kg of body weight in 200mls of 5% dextrose solution over 4 hours, with prophylactic magnesium, potassium, and fluid supplementation. Phenergan and paracetamol are also administered for the associated thrombophlebitis, headache, chills, and drowsiness.
Renal function tests and magnesium levels are performed every three days.

The treatment protocol to be used is attached as Appendix B.

2.4. Sampling technique.

All patients diagnosed and treated for cryptococcal meningitis with amphotericin B at Klerksdorp/Tshepong hospital complex during the period 01/07/2013 to 30/06/2014, who met the inclusion criteria, were included in the study.

The names and file numbers of the study subjects were retrieved from the NHLS data log for the specified hospital and the specified period after obtaining ethics and protocol committee approval for the study. These were forwarded to the head of internal medicine at Tshepong hospital.

Approval for the study was also obtained from the CEO and the clinical manager at Tshepong hospital.
Using the names and file numbers identified, the files were retrieved from the file room at records department of the hospital for analysis and data collection on to the data collection sheet, attached as Appendix A.

2.5. Data collection procedure.

A total of 165 names and file numbers were identified from the NHLS data log for Tshepong hospital which represented the total number of patients who were diagnosed of cryptococcal meningitis during the period 01/07/2013 to 30/06/2014. Data was collected on 165 patients from the NHLS. From the 165 names and file numbers collected, 73 files were retrieved from records which seemed to have sufficient data as specified in the inclusion criteria for the study, but ultimately 53 data collection sheets had enough data for analysis per study protocol.

2.6.Materials and Instruments Used.

The diagnosis was based on cerebrospinal fluid examination from patients with a clinical suspicion of meningitis sent to NHLS for diagnostic analysis. The tests used for the diagnosis of cryptococcal meningitis were cryptococcal latex antigen and the demonstration of the cryptococcal capsule by the India Ink method. Further examination of the cerebrospinal fluid may have shown mononuclear pleocytosis and high protein, but these parameters would be nonspecific for cryptococcal meningitis.

2.7.1. Renal function tests and magnesium.

The machine used by the NHLS for renal function tests was the DXC 800.
The following tests were used to determine these variables:

- Sodium, potassium and chloride: Indirect potentiometric procedure on ion selective electrode.
- Urea: Diacetyl monoxime method.

- Creatinine: Jaffe method.
- Bicarbonate: Phosphoenolpyruvate carboxylase method.
- Magnesium: Calmigite method.

2.7.2. Normal or reference ranges were as follows:

- Sodium: 136-145 mmol/L
- Potassium: 3.5-5.1 mmol/L
- Chloride: 98-107 mmol/L
- Bicarbonate: 23-29mmol/L
- Urea: 1.4-5.4 mmol/L
- Creatinine: 39-85 umol/L
- Magnesium: 0.62-0.91 mmol/L

2.7. Data Analysis.

Mean and standard deviation was used for analysis of non-parametric data with normal distribution, and only medians and range were used for parametric data with abnormal distribution, as well as for non-parametric data.

Survival analysis and the random effects model were used to analyze data, specifically time-related events, dosage relationship with adverse events, and variations between participants.

Comparative analysis was done between those with no pre-existing risk factors for nephrotoxicity or any other nephrotoxic drugs versus those with such risk factors, pre-existing renal dysfunction and those on other nephrotoxic drugs.

p-value was significant at 0.05.

2.8. Ethics

Permission was applied for and obtained a letter from the WITS Ethics Committee before data collection commenced. The letter was also forwarded to the CEO, clinical manager and the head of internal medicine at Tshepong hospital.

2.9.Funding

The whole project was personally funded, and costs did not exceed R5000, 00.

3 CHAPTER THREE: STUDY RESULTS.

3.1. Description of the baseline demographics, anthropometric and clinical features of the patients.

Initially 72 files were reviewed, but the final sample size included only 53 files, as kidney injury status was not recorded in the other patient files. Of the 53 files, the mean CD4 count was 35, while the mean viral load was 735229 copies/1. The ART-regimen of choice for study participants was TDF-containing regimen for those with no renal dysfunction at the time of initiation of the ART. As a result, most of the study participants on ART were on TDF-containing regimen at the start of the study period. The mean weight of the patients was 58.41 (±12.59). The most prevalent comorbidities were bacterial meningitis (empirically treated), in 62% of the patients, sepsis (18%) and tuberculosis (17%).

Figure 3-1 below provides a graphic illustration of the health status of the study participants, with 83% of the patients developing acute kidney injury, and the other 17% had no AKI.

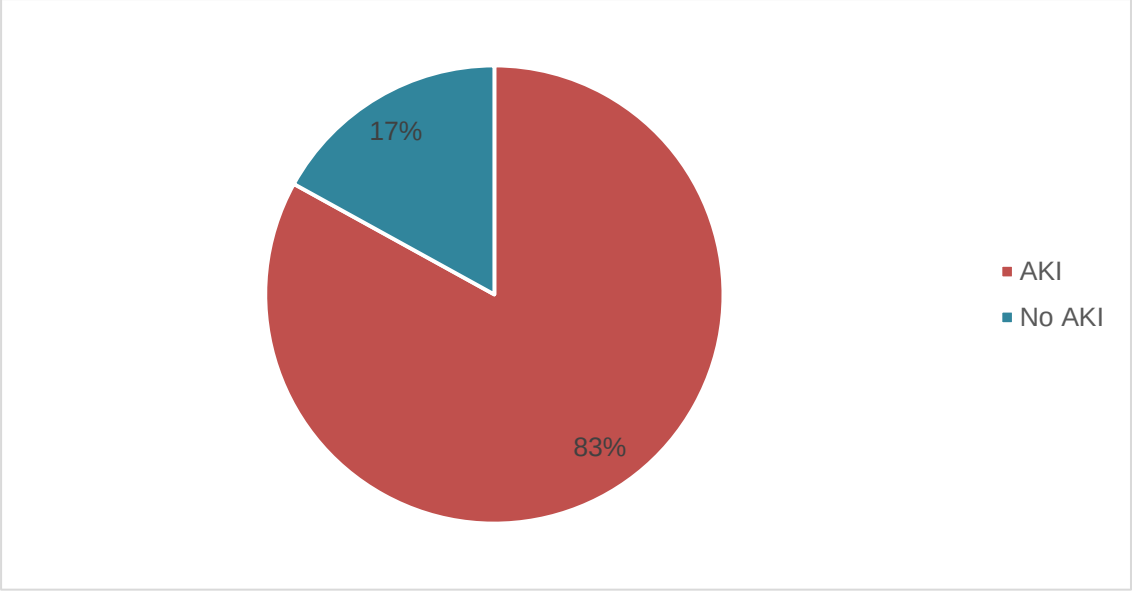

Figure 3-1: Distribution of study participants by health status

Table 3-1: Demographic characteristics of study participants

Characteristics	Frequency (%)
Sex	
Male	28 (52.83)
Female	25 (47.17)
Age	37.79 (9.68)
On ART	35 (66.04)
Not on ART	18 (33.96)
CD4 (n=48)	43.35 (35.96)
Viral Load (n=26)	735229.1 (1125667)
Number of days under Amphotericin (n=44)	12 (12)
Weight (n=33)	58.41 (12.59)
Dose	51.70 (6.65)
Total dose (n=33)	639.43 (187.59)
Baseline Sodium	131.70 (4.54)
Baseline Potassium (n=51)	3.90 (0.61)
Baseline Bicarbonate (n=50)	21.94 (3.91)
Baseline Magnesium (n=8)	0.8 (0.11)
Worst Sodium Level (n=44)	137.18 (5.63)
Worst Potassium (n=43)	4.02 (1.15)
Worst renal function (n=42)	100.5 (23.99)
Worst Urea (n=44)	11.56 (17.11)
Comorbidities	
Tuberculosis	17 (32.08)
Bacterial Meningitis	33 (62.26)
Sepsis	18 (33.96)
Herpes Simplex	2 (3.77)
Convulsion	5 (9.43)
Hypertension	11 (20.75)
Diabetes Mellitus	1 (1.89)
Diarrhoea	3 (5.66)
Epilepsy	1 (1.89)
Gastritis (Peptic Ulcer)	1 (1.89)
Previous cryptococcal meningitis	**15 (21.4)**

Table 3-1 shows that on average the patients were adults with a mean age of 37 years with a standard deviation of about ten years. Males represented 52.83% of the subjects, whereas females represented 47.17%. The term ‘worst’ is used in the cohort to represent the variables when renal dysfunction was most severe for the individual participant.

Table 3-1 also shows that 21.4% of the study participants had previous cryptococcal meningitis which may have resulted from immunological or treatment failure from any cause.

Figure 3.2 below illustrates the distribution of patients by sex.

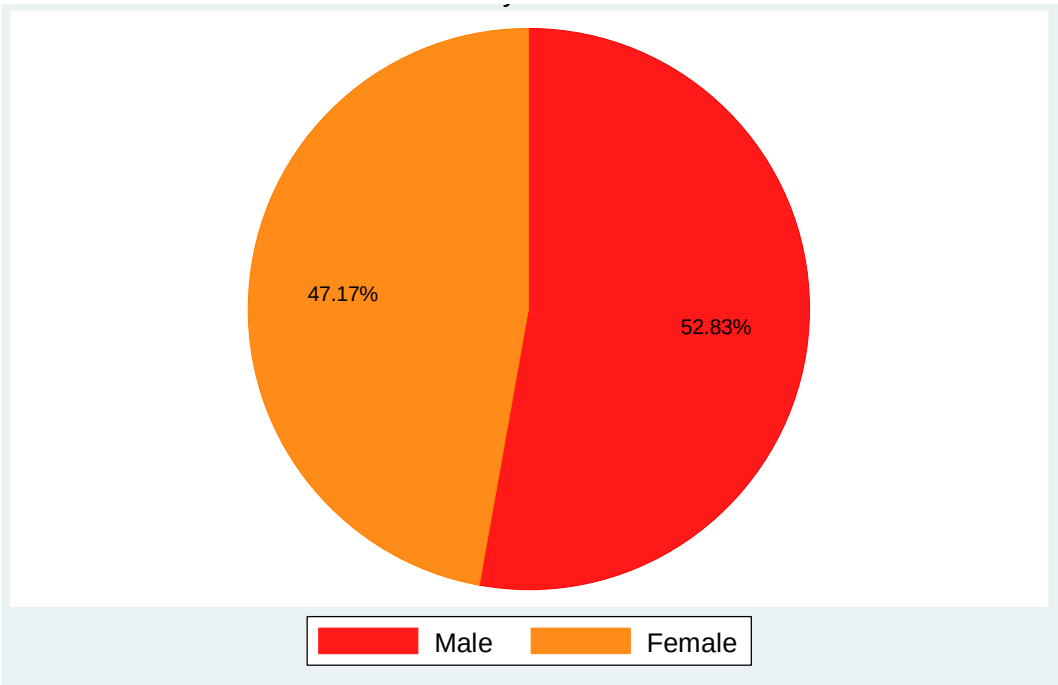

Figure 3-2: Distribution of study participants by sex

Figure 3.3 below show the distribution of study participants by age with the mean of 37.79 and a range of 9.68.

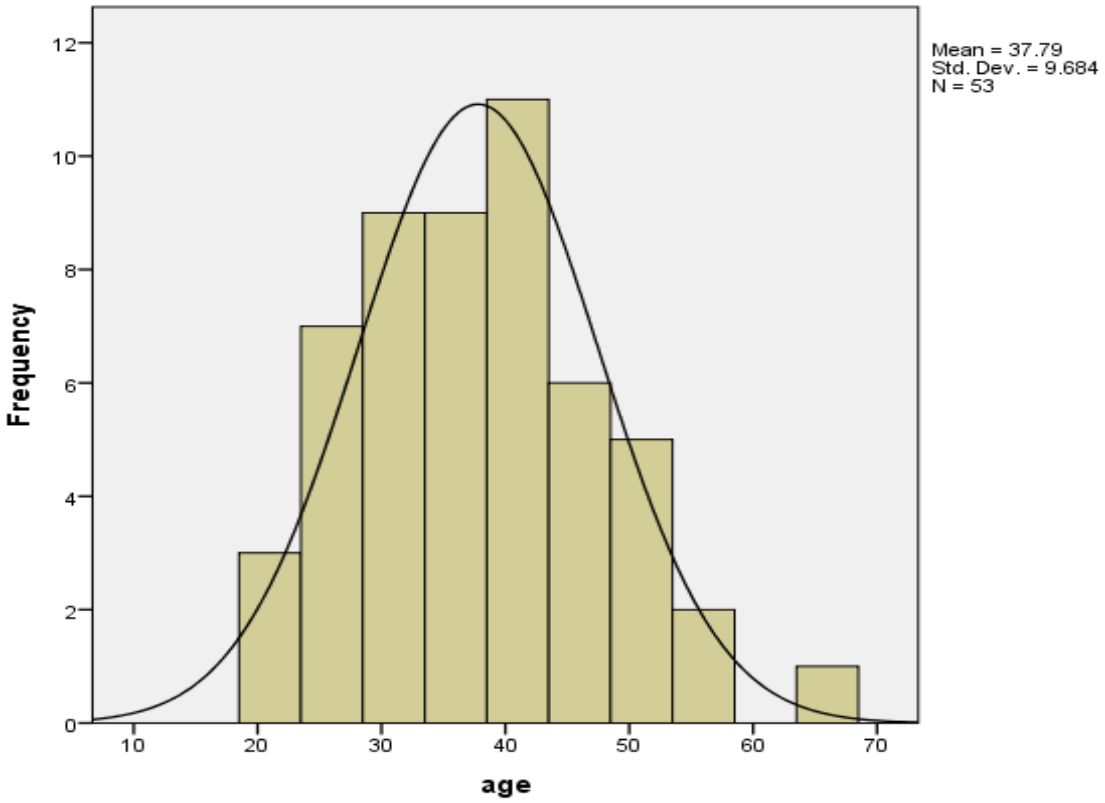

Figure 3-3: Age distribution of the study participants

3.2. Association between socio-demographic, anthropometric and clinical characteristics of the patients and the occurrence of kidney injury

Of the 53 files for which information on the kidney injury status was available, 83% developed acute kidney injury during therapy.

Table 3-2: Association between baseline characteristics and kidney injury

Characteristics	Kidney Injury Status		p-value
	Yes	No	
Sex			
Male	25 (89.29 %)	3 (10.71 %)	0.198
Female	19 (76.00 %)	6 (24.00 %)	
Age	37.79 (9.68 %)	39.22 (8.38 %)	0.63
ART Initiated			
No	12 (66.67 %)	6 (33.33 %)	0.03
Yes	32 (91.43 %)	3 (8.57 %)	
Square root CD4 (n=48)	6.05 (2.82)	5.83 (2.15)	0.083
Viral Load (n=26)	156316 (17717-982885)	268572 (47262-2560494)	0.50
Weight (n=33)	55.35 (47.7-63.5)	53.9 (50.3-54.2)	0.51*
Dose	52.27 (7.03)	48.89 (3.33)	0.07*
Total dose (n=33)	700 (525 - 700)	700 (550 – 700)	0.69
Total dose/Kg (in mg)	11.67 (3.64)	12.51 (3.38)	0.64
Baseline Sodium	132.07 (4.71)	129.88 (3.26)	0.19
Baseline Potassium (n=51)	3.91 (0.61)	3.82 (0.69)	0.69
Baseline Bicarbonate (n=50)	21.63 (3.91)	23.33 (3.84)	0.24
Baseline Magnesium (n=8)	0.8 (0.12)	0.8 (0.1)	1.00
Fluid replacement			
No	5 (71.43)	2 (28.57)	0.59
Yes	39 (84.78)	7 (15.22)	
Comorbidities			
Tuberculosis			
Yes	16 (94.12)	1 (5.88)	0.24F
No	28 (77.78)	8 (22.22)	
Bacterial Meningitis			
Yes	26 (78.79)	7 (21.21)	0.46F
No	18 (90.00)	2 (10.00)	
Sepsis			
Yes	15 (83.33)	3 (16.67)	0.97
No	29 (82.86)	6 (17.14)	
Drugs co-administered			
ceftriaxone			
Yes	30 (78.95)	8 (21.05)	0.21
No	14 (93.33)	1 (6.67)	
cotrimoxazole		**1**	
Yes	25 (92.59)	2 (7.41)	0.08F
No	19 (73.08)	7 (26.92)	
Cloxacillin			
Yes	5 (62.50)	3 (37.50)	0.12
No	39 (86.67)	6 (13.33)	

Pyridoxine			
Yes	16 (88.89)	2 (11.11)	0.701
No	28 (80.000	7 (20.00)	
Metoclopramide			
Yes	10 (71.43)	4 (28.57)	0.18
No	34 (87.18)	5 (12.82)	

Note: For continuous variables independent t-test was used unless otherwise indicated

For categorical variables, Pearson Chi-square test was used unless otherwise indicated

***** *The test refers to Mann-Whitney test for equality of the sum of rank*

Of the selected covariates, only being on ART had a statistically significant association with kidney injury. Dose of amphotericin B, cotrimoxazole, and cloxacillin were marginally significant at 10% level of significance.

Figure 3.4 shows the distribution of age, CD4 count and weight between those who developed kidney injury and those who did not. No significant difference in risk factors are shown in the cohorts.

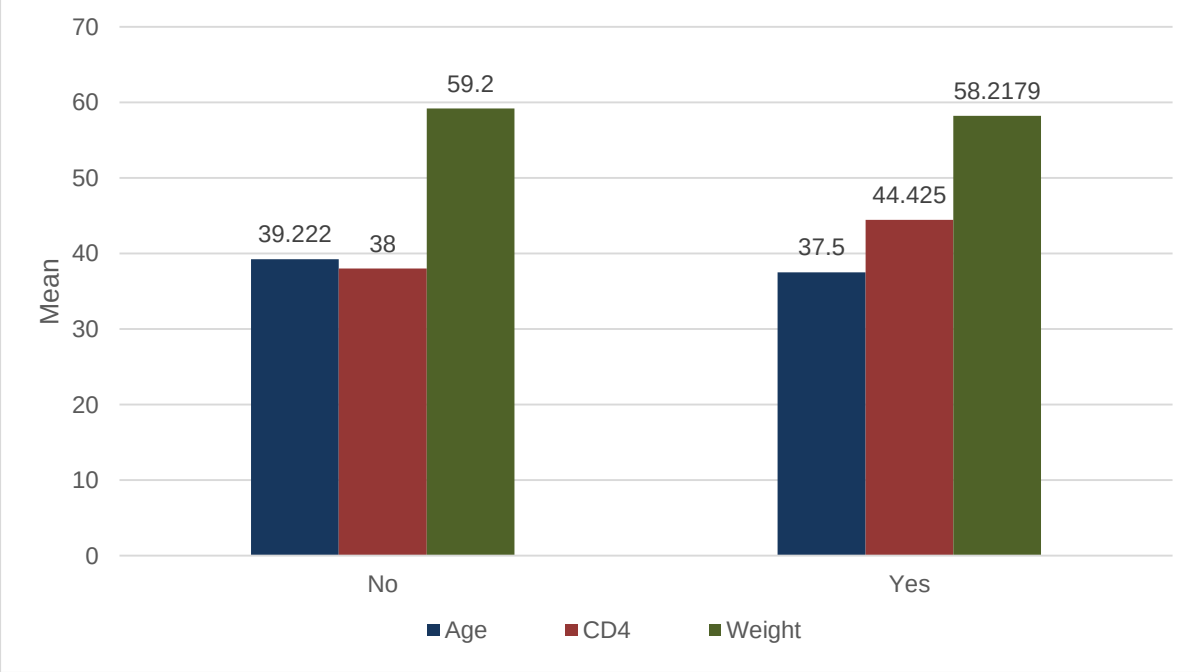

Figure 3-4: Distribution of Age, Weight and CD4 by AKI status

However, being on ART showed a significant association with kidney injury as portrayed in figure 3.5 below (p-value of 0.03).

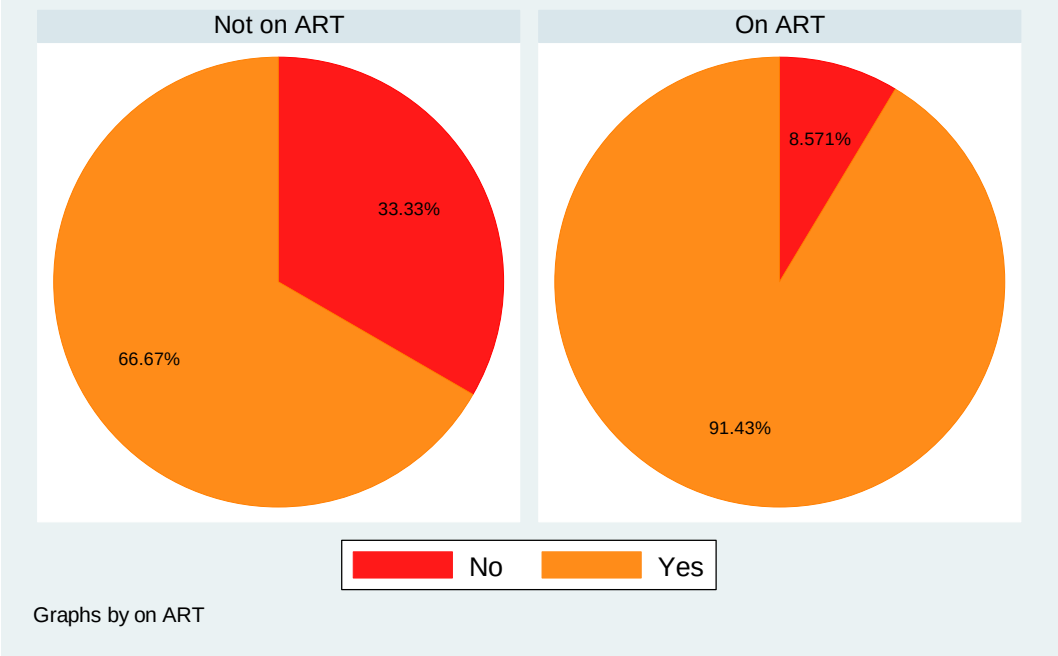

Figure 3-5: Acute kidney injury by ART status

Of the 35 patients who were on ART at baseline, 91.43% had kidney injury whereas of the 18 patients who were not on ART, 66.67% had acute kidney injury with p-value of 0.03.

Table 3-3 below shows that the most frequent drugs co-administered were ceftriaxone (38 times), cotrimoxazole (27 times), and Pyridoxine (18 times).

Table 3-3: Description of common drugs combined with Amphotericin B

Drug name	**Frequency (%)**
Ceftriaxone	38 (71.70)
Dexamethasone	15 (28.30)
Rifafour	15 (28.30)
Tenofovir	9 (16.98)
Odimune(FDC)	8 (15.09)
Co-amoxiclav	9 (16.98)
Cotrimoxazole	27 (50.94)
Enoxaparin	10 (18.87)
Clonazepam	10 (18.87)

Lamivudine	12 (22.64)
Pyridoxine	18 (33.96)
Metoclopramide	14 (26.42)
Hydrochlorothiazide	10 (18.87)
Tramadol	12 (22.64)

Table 3-4: Description of mortality outcomes in association with Acute Kidney Injury

Outcome	**Acute Kidney Injury**		**P-value**
	Yes	**No**	
Mortality			
Yes	9 (90.00)	1 (10.00)	1.00
No	35 (81.40)	8 (18.60)	

Acute kidney injury did not show a statistically significant association with mortality.

No significant association (p-value: 0.11) was found between chronic kidney disease, acute kidney disease and total dose per kg of amphotericin B used. This graphically shown in figure 3.6 below.

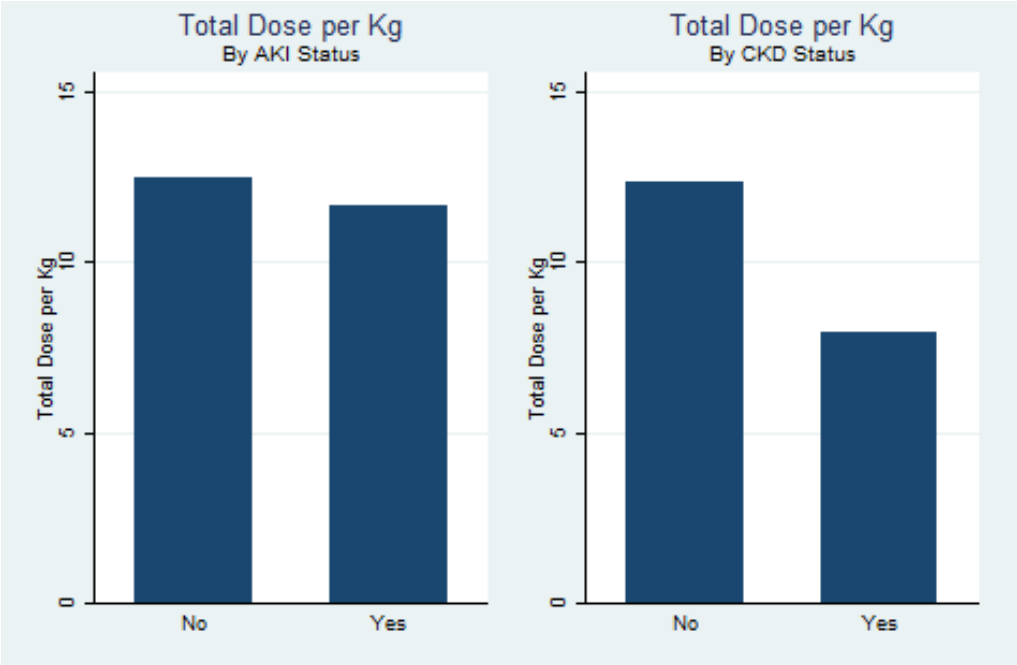

Figure 3-6: Distribution of Total Dose per Kg

3.3. Instantaneous risk of developing acute kidney injury while on Amphotericin B: A survival Analysis using Cox Proportional Hazard Model.

Descriptive survival analysis reveals that on average, patients spent 11 days on Amphotericin B before they failed (developed acute kidney injury); 25% of the patients spent seven days before they developed a kidney injury, whereas the upper 75% quartile spent 14 days. The incidence of developing kidney injury was about 8 per 100 person days.

Table 3-5 below presents results from the Cox proportional hazard model with some of the selected risk factors. Hazard ratios are reported with 95% confidence intervals.

Table 3-5: Cox proportional hazard model

Variable	**Bivariate Cox PH Model**	**Multivariate Cox PH Model**
	HR & 95% C.I	**HR & 95% C.I**
Sex		
Male	1	
Female	0.93 (0.51 – 1.69)	
On ART		
No	1	1
Yes	1.32 (0.67 – 2.59)	1.68 (0.81 – 3.49)
Standard Deviation Dose	1.04 (0.99 – 1.09)	
Baseline Sodium	0.95 (0.90 – 1.00)	0.93 (0.88 – 0.99)
cotrimoxazole		
No	1	
Yes	0.68 (0.37 – 1.27)	
Cloxacillin		
No	1	
Yes	0.74 (0.29 – 1.88)	
Metoclopramide		
No	1	
Yes	0.69 (0.34 – 1.42)	

In the bivariate model, only the baseline serum sodium level was significant at 10% level of significance (p-value 0.06) and dose at 15% level of significance (p-value: 0.13), but

would not be considered significant in my study. The two variables (baseline sodium level and dose) were used selected as the main factors affecting the risk in the multivariate Cox regression model.

In the bivariate model, a unit increase in the level of sodium decreased the risk of kidney injury by 5% (1- 0.95) since (HR: 0.95), whereas a unit increase in dose per kg was more likely to increase the risk of kidney injury by 4% since (HR: 1.04).

In the bivariate analysis, females had about a 7% reduced relative risk of developing acute kidney injury compared to their male counterparts. However, this was not a significant difference (HR: 0.93, 95% C.I: 0.51 – 1.69; p-value: 0.80). As shown below in figure 3.7, the survival curves of the two genders converge at certain points, thereby suggesting that their survival function to acute kidney injury was not different.

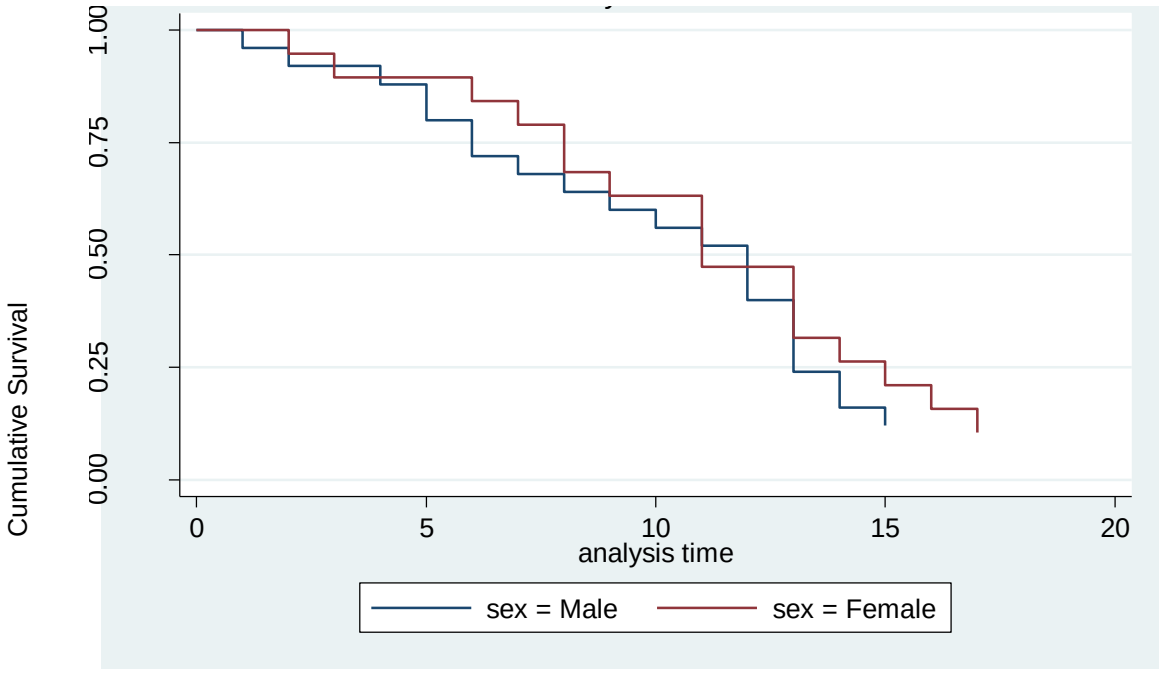

Figure 3-7: Survival function of the patient by gender

Although antiretroviral treatment (ART) initiation showed a significant association in the univariate analysis (see Table 3-2), in the bivariate Cox proportional hazard regression model, there was no significant statistical difference between those on ART and those not on ART. Figure 3.8 below suggests that those on ART at the initiation of amphotericin B developed acute kidney injury faster than those not on ART, but the two curves converge around the 6th day of treatment. However, it should be noted that the two K-M curves

remained parallel and non-convergent for the rest of the time, suggesting that other factors, not captured by the study, may have interplayed to hide such a difference.

Table 3-2 above shows that in the bivariate Cox regression model, cotrimoxazole did not contribute to the development of acute kidney injury. However, such a difference was not statistically significant as shown in figure 3-9 below, with the two survival functions overlapping over time.

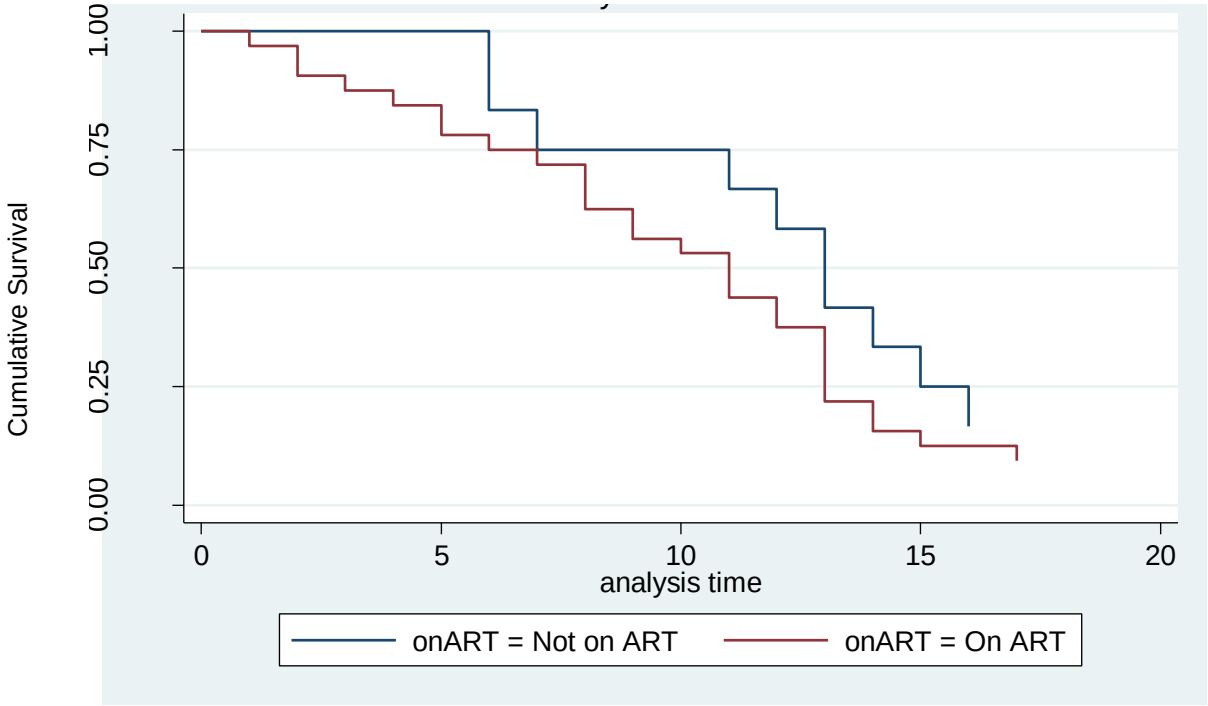

Figure 3-8: Incidence of AKI by ART status

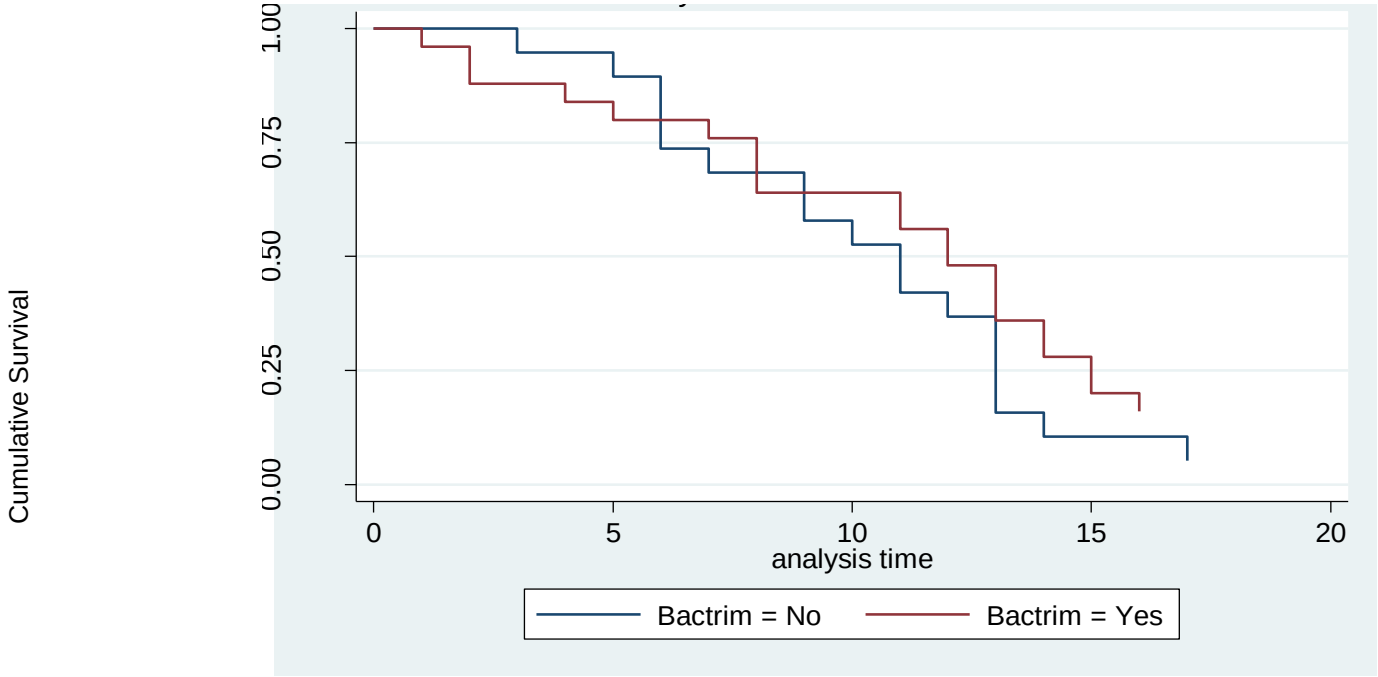

Figure 3-9: Incidence of AKI by administration of cotrimoxazole

My final model was built from considering ART as a primary exposure even though it was not statistically significant in the bivariate Cox regression model. This choice was justified by the fact that I wanted to know if ART combined with Amphotericin may interact negatively to cause acute kidney injury among patients.

Our final best fitting model only included ART initiation status and baseline sodium level. The goodness of fit for this final model was investigated using Cox-Snell residuals. Figure 3-10 below shows that the final model fits the data quite well.

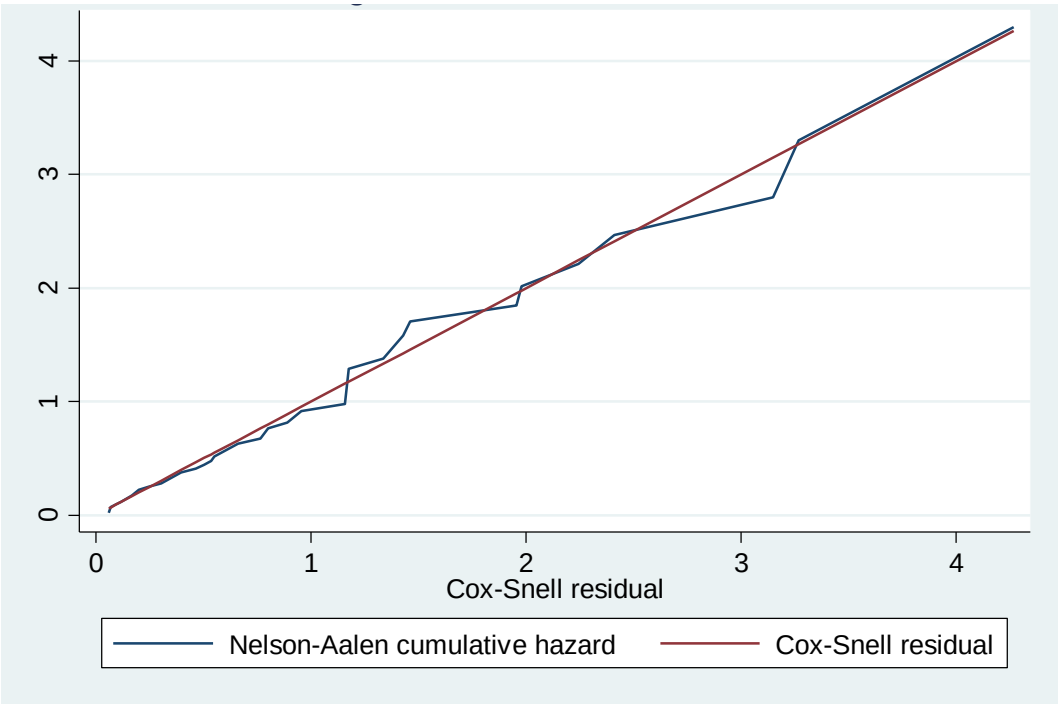

Figure 3-10: Overall goodness of fit

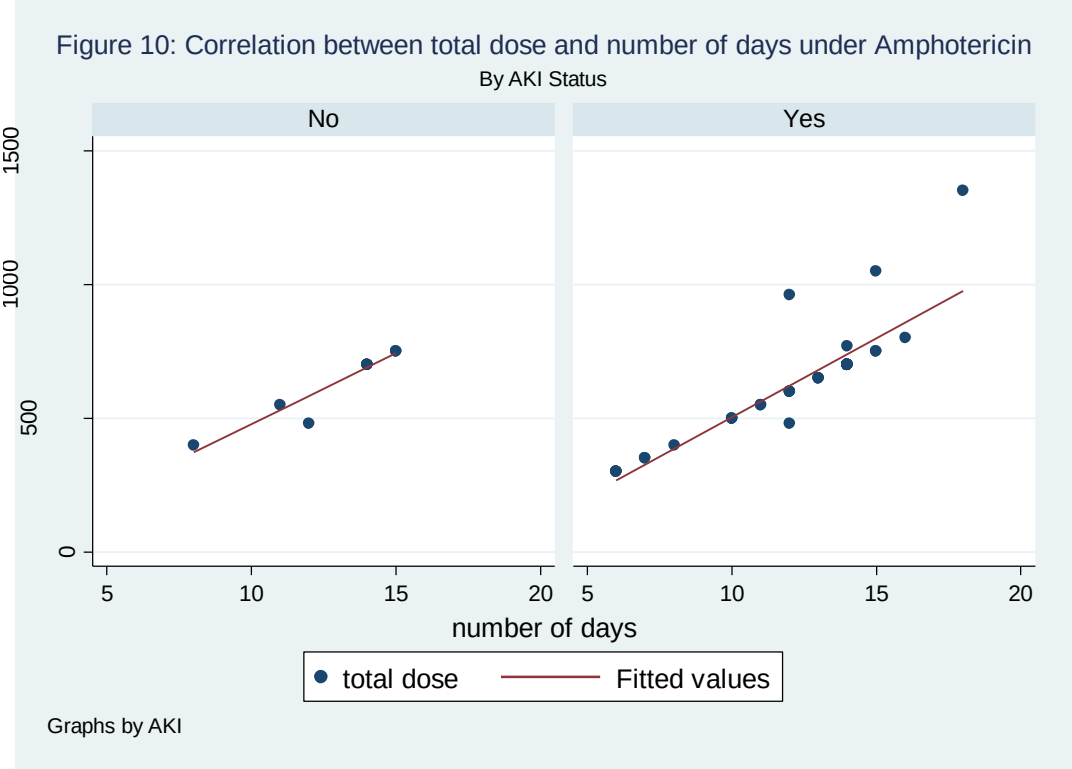

Figure 3-11: Correlation between total dose and number of days under amphotericin B

I further investigated the relationship between total dose received and number of days under amphotericin B among those who developed AKI and those who did not. I

fitted a simple liner regression model, which gave me two different slopes. For those who developed AKI, an incremental day on amphotericin B increased the total dose by 59.01 (std. error: 4.92) whereas for those who did not develop AKI, an additional day under amphotericin increased the total dose by 52.86 (std. error: 6.51). This implies that the higher dose of amphotericin B increases the likelihood of developing acute kidney injury. And this is furthermore supported by figure 3-11 above.

4 CHAPTER FOUR: DISCUSSION.

4.1. Demographics

The study is a retrospective observational review of patients' records for adult patients diagnosed and treated for cryptococcal meningitis at Tshepong Hospital during the period 01 July 2013 to 30 June 2014. Of the 165 names retrieved from NHLS, only 53 had enough data for the study with 28 participants attending follow-up clinic. A significant proportion of 83% developed acute kidney injury with mortality of 18.87% and chronic kidney disease at 5.66%. The study revealed a statistically significant risk associated with being on Tenofovir-containing antiretroviral regimen with 91.43% of those on Tenofovir-containing ART developing AKI. Only 33 of the participants had weight recorded with a positive association between higher doses per kilogram and the development of AKI.

4.2. Results and Discussion

4.2.1 General Discussion

Poor record keeping necessitated a reduction in sample size from the names and file numbers retrieved from the NHLS database, to the actual files with enough data for this study. Most of the files were not found, and some of the files found did not have enough data.

Figure 3-1 on page 18 shows the distribution of study participants by health status. As mentioned in the paragraph above, the majority of the study participants developed AKI (83%) and this was associated with a mortality rate of 18.87% compared to the described mortality of 30 to 40% on amphotericin B-based regimen in other published studies.[12, 16, 21] None of the study participants was offered dialysis. The lower mortality can be attributed to immediately stopping the nephrotoxic drug (Amphotericin B and ART regimen) as soon as AKI develops, good adherence to a treatment protocol, including fluid and electrolyte supplementation, and regular evaluation of renal function tests.

Table 3-1 on page 18 further gives the characteristics of the study participants. Males represented 52.83% of the subjects. The mean age of all participants at 37.79 years of age. All participants were HIV positive with a mean CD4 count of 35 cells/ml, with 66.04% on antiretroviral treatment. This is lower than the well described CD4 of 200 cells/ml, associated with the risk of developing cryptococcal meningitis [4]. A third of the patients with a mean CD4 of 35 cells/ml were not on antiretroviral treatment, which is problematic. This problem is highlighted by the number of study participants with previous cryptococcal meningitis (21.4%), emphasizing the need for better treatment monitoring and compliance with treatment.

The mean number of days on amphotericin B was 12, compared to the 14 days as recommended per published amphotericin B protocols for cryptococcal meningitis. This is because those who developed AKI while on the drug were immediately taken off the drug. The average total dose of amphotericin B received per patient was at 639.43 mg. The average highest sodium level recorded during treatment was at 137 mmol/L compared to average baseline sodium level at 131 mmol/L, while the average highest potassium level was at 4.02 mmol/L (range of 1.15) compared to the average baseline level of 3.9 mmol/L (range of 0.61). This is an important finding as amphotericin B nephrotoxicity is associated with hypokalemia, hyponatraemia and hypomagnesaemia as part of amphotericin B-induced proximal tubulopathy. This can be attributed to supplementation of fluids and electrolytes as per the protocol used (appendix B on page 44), as well as monitoring of renal function.

Hypokalemia, hypercalcemia and phosphate depletion are known to potentiate the toxic effect of nephrotoxic drugs. In this cohort, calcium and magnesium were not routinely monitored and were also not included in the protocol (appendix B) used for the treatment of cryptococcal meningitis with amphotericin B. Only potassium was monitored as part of the renal function tests. As shown in table 3-2 on page 23, the baseline potassium in both the AKI group and those that did not develop AKI were normal with no statistically significant difference between the two groups. The p-value was 0.69.

The data from the study shows that supplementation of fluids and electrolytes, as per protocol, occurred in 79% of subjects for potassium, 77% for magnesium and 90.56% for fluids. Comorbidities are also indicated with bacterial meningitis based on treatment at

62.26% followed by sepsis at 33.96% and tuberculosis at 32.08%. Bacterial meningitis was empirically treated in a large proportion of the study participants, 62.26%, but there was no statistical difference in mortality between those treated for bacterial meningitis and those that were not treated (p- value of 0.46).
Sepsis also did not show any statistically significant contribution to mortality between those treated for sepsis and those that were not treated for sepsis (p- value of 0.97).

Table 3-2 on page 20 shows the breakdown of the study participants by association of baseline characteristics with acute kidney injury, with 83% of the participants having developed acute kidney injury during treatment with amphotericin B, versus those who did not. Of all factors analyzed, including sex, age, baseline renal function tests, and co morbidities, being on HIV treatment which included Tenofovir was the only statistically significant factor contributing to the development of acute kidney injury during treatment with amphotericin B (p-value 0.03). The concomitant use of cotrimoxazole was not significant for the development of AKI in this study (p-value of 0.08).

Figure 3-4 on page 23 shows the distribution by age, weight and CD4 in the proportion of study participants that developed acute kidney injury. This revealed no significant difference between these subgroups suggesting that these factors do not influence the risk of developing acute kidney injury.

Table 3-4 on page 23 shows the comparison of the proportion of mortality in participants who developed acute kidney injury versus those who did not. There is no statistical significance revealed between the subgroups with a p-value of 1.0. The reason for the lack of statistical difference in mortality rate between those participants that developed acute kidney injury versus those that did not is not clear, noting that dialysis was not offered to any of the study participants. However, possibilities include early detection of acute kidney injury and withdrawal of the offending drug, which is amphotericin B in this cohort, and modifying the ART regimen.

Figure 3-6 on page 24 shows the distribution of study participant who developed chronic kidney disease and those who did not in relation to total dose per kilogram body weight. There is no statistical significance with a p-value of 0.11. This suggest that the

progression from acute kidney injury, once established, to chronic kidney injury is not necessarily dose dependent.

Figure 3-6 on page 24 shows the distribution of the study participants with regards to total dose per kg in those who developed acute kidney injury and chronic kidney injury compared to those who did not. The total dose per kg during the whole course of treatment is higher in the group who did not develop acute kidney injury or chronic kidney injury compared to those who did. This is because in those who developed acute kidney injury, the treatment was stopped while those without acute kidney injury continued to complete the course. This also suggests that the daily dose of amphotericin B is important and not just the cumulative dose as illustrated by figure 3-11 on page 30. A small proportion of those who developed acute kidney injury then went on to develop chronic kidney injury.

However, figure 3-11 on page 30 shows that amongst those that developed acute kidney injury, the rate of dose accumulation, the gradient of the graph, was higher in the group that developed acute kidney injury compared to those who did not (59.01 mg/day and 52.86 mg/day). This suggests that the higher the daily dose, the higher the risk of developing acute kidney injury.

A descriptive survival analysis using Cox proportional hazard modelling revealed that on average participants spent 11 days on amphotericin B before developing acute kidney injury, with 25% of study participants developing acute kidney injury at day seven and 75% of study participants developing acute kidney injury by the 14^{th} day. The incidence of developing acute kidney injury was 8 per 100 person days on amphotericin B. In the bivariate model, a unit increase in the level of sodium decreased the risk of kidney injury by 0.95 (HR: 0.95), whereas a unit increase in dose per kg was more likely to increase the risk of kidney injury by only 4% (HR: 1.04). The higher sodium level may be reflective of the use of normal saline (0.9% sodium chloride) use as intravenous fluid during the study or dehydration.

In the bivariate analysis, females had about 7% reduced risk of developing acute kidney injury compared to their male counterparts. However, this was not a significant difference (HR: 0.93, 95% C.I: 0.51 – 1.69; p-value: 0.80). As shown in figure 3-7 on

page 27, the survival curves of the two genders converge at certain points, thereby suggesting that their survival function in terms of acute kidney injury was not different.

Although antiretroviral treatment (ART) initiation showed a significant association in the univariate analysis (see Table 3-2 on page 24), under the bivariate Cox proportional hazard regression model, it showed no statistically significant difference between those on antiretroviral treatment and those who were not on ART. This is better illustrated by figure 3-8 on page 30, which shows that those on ART at the initiation of amphotericin B were getting acute kidney injury faster compared to those who were not yet on ART, yet the two curves converge around the 6^{th} day under treatment. This finding suggests that co-administering the two nephrotoxic drugs increased the risk of developing acute kidney injury. However, it should be noted that the two K-M curves remained parallel and non-convergent for the rest of the time, suggesting that other factors, not captured by the study, may have interplayed to hide such a difference.

4.2.2 Limitations

Record keeping was the major limitation of the study. This includes making good notes by the treating clinician and keeping a good record of investigations done in the file. However, the biggest challenge with record keeping was found in the filing room, with most files not found at all, and only a small fraction having a duplicate file, sometimes with not enough information for the study. NHLS records also did not have all records of investigations done, as some were lost during the system upgrade.

This problem was addressed partially by extending the study period to recruit more patients with records with enough data, as suggested during the protocol assessment meeting.

4.2.3 Implications

The importance of good record keeping by all concerned cannot be over emphasized, including good clinical notes in patients' files to make research easier, simper and effective.

Amphotericin B is a nephrotoxic drug. As a result, it is important to follow administration protocols strictly including fluid and electrolyte supplementation, calculating doses per kg and regular monitoring of renal function and electrolytes.

The co-administration of amphotericin B and Tenofovir significantly increases the already high risk of developing nephrotoxicity.

Follow-up of these patients, especially those with evidence of renal dysfunction during the course of treatment also needs more attention.

The need for renal replacement therapy in patients receiving amphotericin B, who develop acute kidney injury, should be considered where appropriate.

4.2.5 Recommendations.

Amphotericin B is a nephrotoxic drug. As a result, treatment protocols and recommendations should be adhered to as strictly as possible to limit this adverse outcome and improve treatment success rates. Such treatment protocols should include monitoring and correcting electrolyte abnormalities such as hypercalcemia, hypokalemia and hypophosphatemia, that may potentiate the nephrotoxic effects of amphotericin B.

Co-administration of nephrotoxic drugs such as Tenofovir with amphotericin B should be considered with great caution if necessary and cannot be avoided.

More attention to patient education to improve adherence and ultimately patient outcome.

Improved record keeping will enable more detailed future studies also to include urine output, glomerular filtration rate and a better understanding of the relationship between dose and weight. This will also give power to the study to affect change towards safer treatment options and practices. These options include adding flucytosine to reduce the

dose of amphotericin B used, and therefore, the risk of developing AKI on the drug. Lipid formulations of amphotericin B are associated with lower risk of nephrotoxicity and can be used in patients with renal failure.

Future studies which include kidney biopsies to look closer at the histopathological changes associated with the amphotericin B will also help enrich our understanding of the disease and its treatment-related complications in our context.

4.3. Conclusion.

The aim of this study was to describe nephrotoxicity in terms of incidence and outcome in patients with cryptococcal meningitis at Klerksdorp/Tshepong Hospital Complex treated with Amphotericin B administered per local protocol. The study revealed:

- Amphotericin B is nephrotoxic with 83% of the study participants developing acute kidney injury while on treatment with 5.66% progressing to chronic kidney injury.
- The incidence of developing acute kidney injury was at 8 per 100 person days on therapy.
- There was no statistically significant association of the risk of developing acute kidney injury during treatment and mortality, which was at 18.87% compared with 30-40% according to literature without renal dialysis which suggest that adhering to the protocol (appendix B on page 45) used is vital.
- Tenofovir-containing ART regimen had a statistically significant contributor to the risk of developing acute kidney injury on Amphotericin B in this cohort with 91.43% of those on ART developing acute kidney injury.
- There was no statistically significant difference in potassium level in the acute kidney injury group and those without kidney injury, with 79% of the study participants having received potassium supplementation as recommended per amphotericin B treatment protocol (appendix B on page 45).
- Males contributed the majority of the participants at 52.83% with the average age of subjects at 37 years of age.

- None of the study participants was offered renal replacement therapy.

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6 APPENDICES

6.1. Appendix A: Data Collection Form

Allocated patient number: Age: Prev. CCM:

HIV status: CD4/date: VL/date: ART/date:

Default/Date: Restart date:

weight:

Age: Discharge date:

Date of death:

Amphotericin start date: Completion/stoppage date:

Number of dosages or days missed: total dose given:

Other concurrent drugs:

1.
2.
3.
4.
5.
6.
7.
8.

Renal Function tests:

	Base line.	Worst.	Normal post ampho.	Last.
Date.				
Na.				
K.				
Co2.				
Cl.				
Urea.				
Creat.				

Mg.				
KDIGO stage.				

Protocol adhered to: fluid preload.

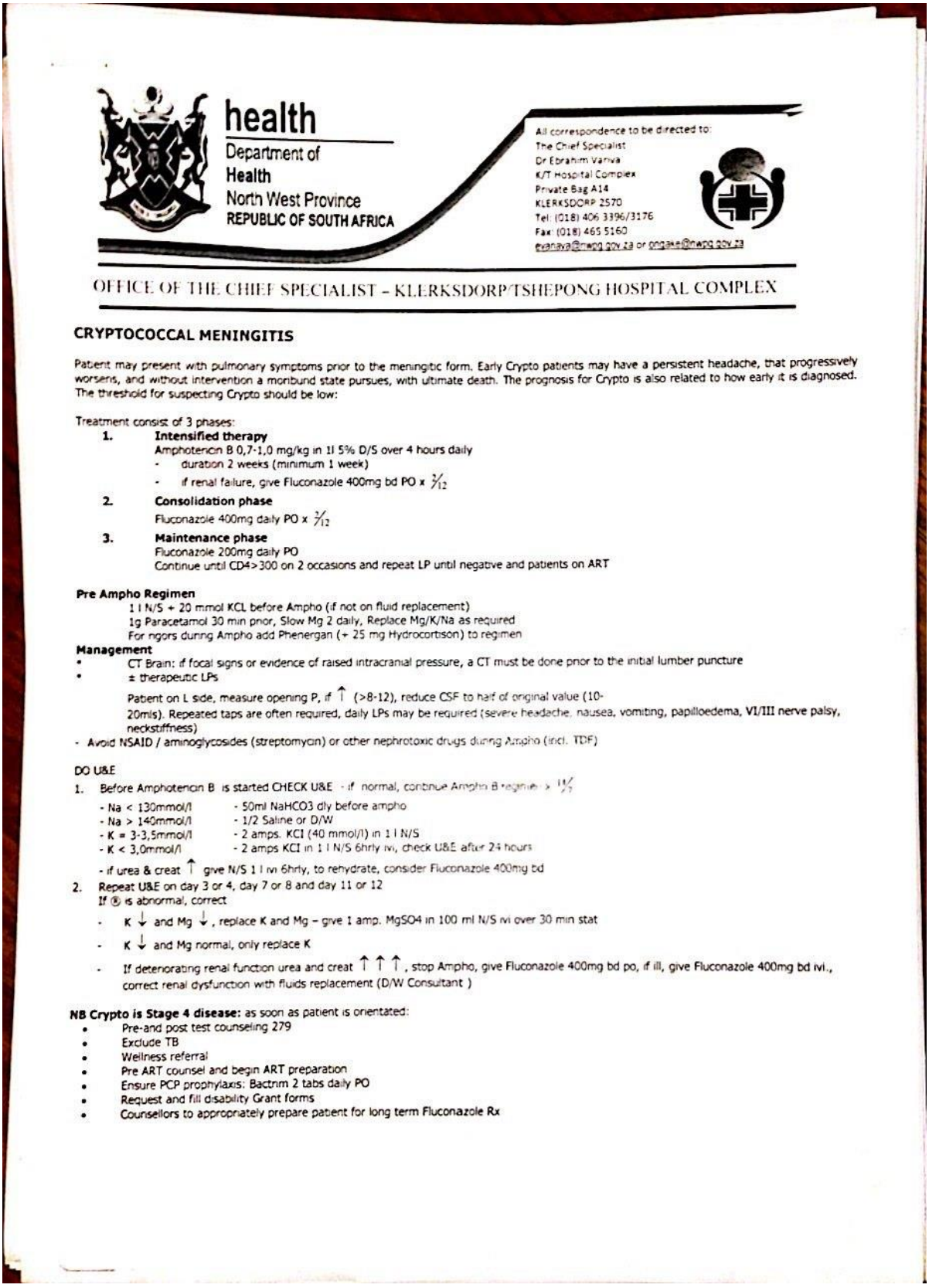
K supplement.

Mg supplement.

Outcome:

- Normal renal functions on completion of ampho.
- Normal renal functions on discharge.
- Dialysed.
- CKD.
- Death.
- Complications:

6.2. Appendix B: Tshephong/Klerksdorp Hospital Complex protocol for the management of cryptococcal meningitis



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OFFICE OF THE CHIEF SPECIALIST – KLERKSDORP/TSHEPONG HOSPITAL COMPLEX

CRYPTOCOCCAL MENINGITIS

Patient may present with pulmonary symptoms prior to the meningitic form. Early Crypto patients may have a persistent headache, that progressively worsens, and without intervention a moribund state pursues, with ultimate death. The prognosis for Crypto is also related to how early it is diagnosed. The threshold for suspecting Crypto should be low:

Treatment consist of 3 phases:

1. **Intensified therapy**
   Amphotericin B 0,7-1,0 mg/kg in 1l 5% D/S over 4 hours daily
   - duration 2 weeks (minimum 1 week)
   - if renal failure, give Fluconazole 400mg bd PO x 3/12
2. **Consolidation phase**
   Fluconazole 400mg daily PO x 3/12
3. **Maintenance phase**
   Fluconazole 200mg daily PO
   Continue until CD4>300 on 2 occasions and repeat LP until negative and patients on ART

Pre Ampho Regimen
1 l N/S + 20 mmol KCL before Ampho (if not on fluid replacement)
1g Paracetamol 30 min prior, Slow Mg 2 daily, Replace Mg/K/Na as required
For rigors during Ampho add Phenergan (+ 25 mg Hydrocortison) to regimen

Management
- CT Brain: if focal signs or evidence of raised intracranial pressure, a CT must be done prior to the initial lumber puncture
- ± therapeutic LPs

  Patient on L side, measure opening P, if ↑ (>8-12), reduce CSF to half of original value (10-20mls). Repeated taps are often required, daily LPs may be required (severe headache, nausea, vomiting, papilloedema, VI/III nerve palsy, neckstiffness)

- Avoid NSAID / aminoglycosides (streptomycin) or other nephrotoxic drugs during Ampho (incl. TDF)

DO U&E

1. Before Amphotericin B is started CHECK U&E - if normal, continue Ampho B regimen x 1/52
   - Na < 130mmol/l - 50ml NaHCO3 dly before ampho
   - Na > 140mmol/l - 1/2 Saline or D/W
   - K = 3-3,5mmol/l - 2 amps. KCl (40 mmol/l) in 1 l N/S
   - K < 3,0mmol/l - 2 amps KCl in 1 l N/S 6hrly ivi, check U&E after 24 hours
   - if urea & creat ↑ give N/S 1 l ivi 6hrly, to rehydrate, consider Fluconazole 400mg bd
2. Repeat U&E on day 3 or 4, day 7 or 8 and day 11 or 12
   If ® is abnormal, correct
   - K ↓ and Mg ↓, replace K and Mg – give 1 amp. MgSO4 in 100 ml N/S ivi over 30 min stat
   - K ↓ and Mg normal, only replace K
   - If deteriorating renal function urea and creat ↑ ↑ ↑, stop Ampho, give Fluconazole 400mg bd po, if ill, give Fluconazole 400mg bd ivi., correct renal dysfunction with fluids replacement (D/W Consultant)

NB Crypto is Stage 4 disease: as soon as patient is orientated:
- Pre-and post test counseling 279
- Exclude TB
- Wellness referral
- Pre ART counsel and begin ART preparation
- Ensure PCP prophylaxis: Bactrim 2 tabs daily PO
- Request and fill disability Grant forms
- Counsellors to appropriately prepare patient for long term Fluconazole Rx

6.3. Appendix C: ETHICS CLEARANCE

R14/49 Dr Kafofora Gerald Maroka

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M141193

NAME: (Principal Investigator)	Dr Kafofora Gerald Maroka
DEPARTMENT:	Internal Medicine Klerksdorp/tshepong Hospital Complex
PROJECT TITLE:	Amphotericin B Nephrotoxicity in Patients with Cryptococcal Meningitis
DATE CONSIDERED:	28/11/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Graham Paget
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	17/11/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in May and will therefore be due in the month of May each year.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES